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Examination of Factors That Influence the Expansion of the Fragile X Mutation in a Sample of Conceptuses From Known Carrier Females

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The Collaborative Prospective Fragile X Study was established to collect information on the pregnancy outcome of women known to be carriers of the fragile X syndrome. The prospective design of this study allows collection of ascertainment-free data and, thereby, avoids biases caused by sampling problems encountered in retrospective family studies. The results of 337 submitted cases are summarized. These data show that the segregation of the fragile X mutation is normal and the sex ratio of conceptuses is as expected for a prenatal sample. There is no excess of dizygotic twinning among the pre- or full mutation carrier females. Data are limited at this time but provide a suggestion that the risk of expansion to the full mutation may be correlated with maternal age and to the parental origin of premutation of carrier women. More data are needed to confirm these suggested trends. The prospective data base provides a valuable resource to continue to examine factors in an unbiased fashion.

KEY WORDS: fragile X, repeat sequence disorder, expansion, mutation

INTRODUCTION

To date, at least seven disorders have been identified as having a dynamic trinucleotide repeat mutation, one of most frequent being the fragile X syndrome. In all of these disorders, the manifestation and/or severity of symptoms are related to the increased number of trinucleotide repeats found in the specific region of the gene. The increase in number of repeats occurs in each subsequent generation once the repeat sequence is unstable. Although the risk to expand has been quantified, the actual mechanism of expansion is unknown. The transmission of the fragile X mental retardation gene (FMR1) can be used as a model to characterize the underlying process of expansion and some of the factors that influence it.

The Collaborative Prospective Fragile X Study was initiated in the beginning of 1991 to collect information on the pregnancy outcome of women known to be carriers of the fragile X syndrome [Sherman, 1991]. The information includes phenotype, cytogenetic data on fragile X site expression, and molecular assessment of number of repeats. The prospective design of this study allows collection of ascertainment-free data and, thereby, avoids biases caused by sampling problems encountered in retrospective family studies. Our previous report summarized the DNA results on the first 152 cases of female carriers and their pregnancy outcomes [Sherman and contributing authors, 1994]. Over 300 cases have now been submitted and the results are summarized.

MATERIALS AND METHODS

In the data presented, the unit of study is the pregnancy outcome of a female known to carry the fragile X mutation. Data on male carriers are too limited for analysis as yet. The pregnancy outcome includes prenatal diagnoses and live births. An individual is eligible for study if he or she is known to be a carrier of the fragile X mutation prior to the presentation of their pregnancy. Data are submitted by each collaborator using a standardized data collection form.

The minimum data required for case submission included a pedigree and results of DNA studies to identify the repeat sequence mutation. The pedigree of the eligible case was used to establish the prospective nature of ascertainment and to document the family history of the fragile X syndrome. DNA studies were most often based on Southern blot assays and were reported as a delta value, or the difference in size of the fragment containing the repeat sequence between normal individuals and those with the fragile X mutation. Conversion to repeat number was crude and based on the assumption that most individuals in the general population have ap-

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TABLE I. CCG Repeat Number of Known Carrier Females in the Study

FMR1 mutation	No. of cases	Percentage
Pre-mutation carrier		
50–59	10	3
60–69	21	6
70–79	27	8
80–89	37	11
90–99	27	8
100–120	73	22
Unknown size	39	11
Full mutation carriers	103	31
Total	337	100

proximately 29 repeats. Thus, the delta value was divided by 3 (three base pairs in the sequence) and added to 29 repeats. If results based on PCR studies were available, they were used instead of the Southern blot results, as the resolution for premutation carriers is higher.

RESULTS

To date, 370 prospective cases were submitted by collaborators and most are based on prenatal diagnoses. Full information on the CGG repeat number (PCR or Southern blot) of both carrier mother and prenatal diagnosis was available on 337 cases. From these data, it can be estimated that approximately 70% of women who seek prenatal diagnosis for the fragile X syndrome carry the premutation; their repeat sizes ranging from 50 to 120 repeats (Table I). The remaining 30% carry the full mutation.

The segregation of the fragile X mutation and the sex ratio of the conceptuses were not different from expected (Table II). Thus, there is no evidence for selection against zygotes with full mutations. We also examined the rate of multiple births, specifically the rate of dizygotic (DZ) twinning among carrier females of the fragile X mutation. No increased twinning rate was observed among pre- or full mutation carriers (Table III).

We examined two possible factors that may influence the rate of expansion of the premutation to the full mutation in addition to repeat number: maternal age and parental origin of the FMR1 gene of premutation mothers (Table IV). Only premutation mothers who transmitted the mutation to their conceptus were eligible for these analyses. Moreover, only premutation carriers with less than 90 repeats provide information on differential risks to expand to the full mutation; those with 90 or greater repeats almost always expand to the full mutation in their conceptuses (Table V). Thus, the sample sizes are small at this time and comparisons do not show significance; however, the trends are interesting. First, there is a suggestion that older mothers may have a higher risk for expansion to the full mutation compared with younger women. Second, those premutation carrier females who received the mutation from their fathers may have a higher risk compared with those who received the mutation from their mothers. Lastly, we examined the risk of expanding to the full mutation based on the premutation carrier female's repeat size (Table V). These data confirm the well-established observation that the risk to expand to the full mutation is correlated with increasing repeat number of the carrier female.

DISCUSSION

This ascertainment-free data base of transmission of the fragile X mutation from known carrier females to their conceptuses is a valuable resource to examine features of the expansion of the mutation and factors that influence its behavior. First, the fragile X mutation appears to be transmitted to 50% of all conceptuses as expected. A normal sex ratio indicates that there is no strong selection against male or female conceptuses with the full mutation. In the analysis of the first 152 cases [Sherman and contributing authors, 1994], there was a significant association between the form of the mutation (pre or full mutation) in carrier mothers and the frequency of its transmission. Examination of the segregation ratios (the number of transmissions of the fragile X mutation/total number of transmissions) from premutation mothers showed that there was a deficit of conceptuses that received the fragile X mutation. No deficit was observed from full mutation carrier mothers. In this updated data set, only a trend was seen. That is, the segregation ratio was 0.47 for premutation

TABLE II. Description of Conceptuses of Known Fragile X Carrier Females

	Conceptus carrier status			Segregation ratio[b]	Sex ratio (m:f)
	Non	Pre	Full		
A. Pre-mutation carrier mothers (n = 225)[a]					
Male	65	10	51	0.48	
Female	55	12	32	0.44	
Total	120	22	83	0.47	1.3
B. Full mutation carrier mothers (n = 103)					
Male	27	1	30	0.53	
Female	22	1	22	0.51	
Total	49	2	52	0.52	1.3

[a] Nine cases are excluded due to unknown gender.
[b] Segregation ratio is defined as the number of transmissions of the fragile X mutation/total number of transmissions.

TABLE III. Rate of Dizygotic (DZ) Twinning Among Pre- and Full Mutation Carrier Females

Carrier mother	DZ births among all births	Twinning rate
Premutation	3/231	0.01
Full mutation	2/101	0.02

carriers and 0.52 for full mutation carriers. The most probable explanation is that small premutations were missed, as only Southern blot techniques were used in the first set of submitted cases. Subsequently, more laboratories who were contributing cases are now using PCR methods as an initial study of the conceptus, followed by Southern blot techniques for confirmation. Under this protocol, small premutations are identified.

With respect to pregnancy outcomes, we examined the rate of twinning among the conceptuses of female carriers. The motivation for this analysis was a recent report of increased DZ twinning rates among premutation carriers [Turner et al., 1994]. In their study, it was found that the DZ twinning rate was 1:58 among noncarriers and 1:55 among full mutation carriers (n = 173 and n = 55, respectively) whereas the rate among premutation carriers was as high as 1:11 (n = 253). To confirm this finding, we examined the DZ twinning rate among the prenatal diagnoses of pre- and full mutation carriers. No difference in rate of twinning was found among pre- and full mutation carriers. Furthermore, the observed DZ twinning rate was found to be similar to that expected in a predominantly Caucasian population. At this time, it is difficult to explain the differences between these two studies. In a previous study of twinning, we compared the rate of twins found in fragile X pedigrees with that found in pedigrees with hemophilia instead of population statistics [Sherman et al., 1988]. This was done to adjust for ascertainment of large pedigrees. We found no excess of twins using this comparison. Thus, the reported twin rates of Turner et al. [1994] may be spuriously high due to the type of data analyzed. We will be able to assess these rates further as the prospective data base grows. It is important to assess

TABLE IV. Comparison of the Risk of the Premutation to Expand to the Full Mutation by Maternal Age and Parental Origin of Premutation Among Carrier Females

Repeat number of mother	Risk of expansion to full mutation by maternal age		Risk ratio (old:yng)
	< 30 yrs	≥ 30 yrs	
A. Maternal age			
<90	4/13	13/25	1.69
≥90	10/10	19/20	0.95
Repeat number of mother	**Risk of expansion to full mutation by parental origin**		**Risk ratio (pat:mat)**
	Paternal	Maternal	
B. Parental origin of premutation allele			
<90	8/13	5/13	1.60
≥90	16/16	14/14	1.00

TABLE V. Comparison of the Risk of the Premutation to Expand to the Full Mutation by the Repeat Number of the Carrier Female

Repeat number of mother	No. of cases	Risk of expansion
50–59	6	0.00
60–69	8	0.37
70–79	11	0.91
80–89	16	0.63
≥90	39	0.98

carefully this finding, as it suggests that a specific phenotype associated with multiple ovulations may be related to an increased number of CGG repeats in the FMR1 gene, not to the absence of the gene product.

Two factors that influence the size of expansion are now well-established for the fragile X mutation: gender and repeat number of the carrier parent. The repeat number remains relatively stable when transmitted by a premutation father as compared with a premutation mother. Moreover, about 30% of the time, contractions in repeat number occur when the premutation is transmitted by the father [Fisch et al., 1995], in contrast to relatively few among female transmissions. Furthermore, repeat size of the carrier mother is positively correlated with the size of expansion in her offspring: the larger the number of repeats in the premutation mother, the higher the risk of expanding to the full mutation in her offspring [Fu et al., 1991; Yu et al., 1992; Heitz et al., 1992; Snow et al., 1993]. Recent data suggest that AGG interspersion among the CGG repeat sequences is important in determining initial instability [Kunst and Warren, 1994; Eichler et al., 1994; Snow et al., 1994; Hirst et al., 1994; Zhong et al., 1995]. However, the CGG-AGG pattern is probably not an important property in the expansion process once the number of repeats is greater than 60 repeats. Other factors such as parental age and parental origin of the mutation among carrier females have not been examined to determine if they have any effect on expansion rates or sizes. We examined these two factors in this prospective data base of transmissions from known carrier females to their conceptuses.

It is important to examine parental-age effects for any process that occurs meiotically and shows differences between maternal and paternal transmissions. The expansion process of all the repeat sequence mutations must have at least one component that acts either during pre-meiotic mitotic divisions or during meiosis. This must be assumed to explain the increased risk and/or severity of the disorder in each subsequent generation. This assumption does not contradict the data that suggest that expansion also may occur during early embryonic development [Wohrle et al., 1993; Reyniers et al., 1993; Devys et al., 1992]. Depending on the timing of expansion in meiosis, it may be expected that parental age is associated with the rate of expansion. If expansion occurs during pre-meiotic mitotic divisions, a paternal age effect may be expected, since there are many more mitotic divisions during the formation of sperm compared with eggs. This type of association has been observed among premutation to full mutation expansions of paternal alleles of the Huntington disease

gene [Goldberg et al., 1993]. Alternatively, a maternal age effect may be expected if expansion occurs during prophase of meiosis I, the phase in which eggs are arrested until ovulation. One study of fragile X maternal transmissions compared the size of expansion among 33 full mutation affected sibs and found that the younger sib had a larger repeat sequence than the older sib [Mornet et al., 1993]. There are two explanations for this finding: 1) there is selection against larger sequences in blood over time in an individual; or 2) there is a correlation with maternal age and the size of expansion in her offspring. The prospective study will be ideal to provide data to resolve these alternatives, as all diagnoses are performed at one time point, during the prenatal period. Moreover, results from this analysis will provide evidence to pinpoint the timing of the expansion process during meiosis. Preliminary evidence points to an association with maternal age, although data are too limited to draw any definitive conclusions.

Another factor that may influence rate of expansion is parental origin of the premutation. Certainly, there is a large difference in rates of expansion when the repeat sequence is passed from a male compared with a female. It could be hypothesized that there also may be a difference in expansion rates among premutation females who received their FMR1 gene from their fathers compared to their mothers. Indeed, this is predicted from the models that assume two time points of expansion, during meiosis and during early embryonic development [Ashley and Sherman, 1995], as such models require the assumption that early embryonic expansion is restricted to maternal alleles. Thus, daughters of carrier fathers have similar repeat sizes in their gametes and somatic cells, since paternally inherited alleles are assumed not to undergo mitotic expansion. In contrast, daughters of carrier mothers have smaller repeat sizes in their gametes compared with their somatic cells. Thus, females who received the gene from their father may be at higher risk for having a full mutation offspring than those who received the gene from their mother, assuming the same repeat size is observed in blood among each type of female. The results from the test of this hypothesis are important to distinguish between expansion models. Again, the data are too limited at this point, but suggest that the risk is higher among mothers who received their premutation allele from their fathers.

In summary, it is now well-established that the segregation of the fragile X mutation is normal and the sex ratio of conceptuses is as expected for a prenatal sample. More data are needed to confirm the suggested trends related to factors that influence the rate of expansion of the premutation in females. The prospective data base provides a valuable resource to continue to examine factors in an unbiased fashion.

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